United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health and Science

SOP No: **CLG – RAC1.00**		Page 1 of 22
Title: **Determination of Ractopamine Hydrochloride in Swine and Bovine Liver and Muscle by High Performance Liquid Chromatography**.		
Revision: **.00**	Replaces: NA	Effective: 05/08/03

Contents

Approval signatures on file

A. INTRODUCTION

1. Theory

   Ractopamine, β-adrenergic agonist, is extracted from swine and bovine liver and muscle with methanol. An aliquot of the extract is evaporated, borate buffer is added, and ractopamine is extracted into ethyl acetate by liquid/liquid partition. The sample is purified by using acidic alumina solid phase extraction. The final sample is dissolved in dilute acetic acid and analyzed for ractopamine using high performance liquid chromatography (HPLC) with fluorescence detection.

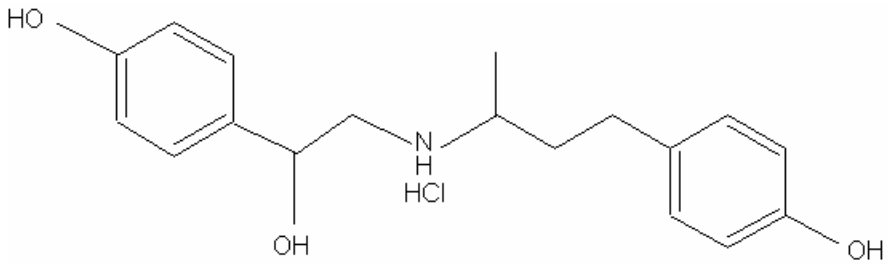
2. Applicability

   This method is suitable to determine ractopamine in swine and bovine liver and muscle tissues.

3. Structure

   Ractopamine HCl is a mixture of four stereoisomers in approximately equal proportions (RS, SR, RR, and SS). This HPLC method does not distinguish between these stereoisomers, and thus results in a single peak for all four stereoisomers present in a particular sample.

Structure of ractopamine HCl.



B. EQUIPMENT

1. Apparatus

   Note: Equivalent instrument and apparatus may be substituted.

   a. HPLC pump - Hewlett Packard 1050 series quaternary pump.

   b. HPLC autosampler - Hewlett Packard 1050 series.

   c. HPLC variable wavelength fluorescence detector - Hewlett Packard 1046A.

   d. HPLC detector recording device - Hewlett Packard 3396 Series III Integrator. Agilent Technologies, Chemical Analysis Group, North American Sales, Customer Contact Center, 2850 Centerville Road, Wilmington, Delaware 19808, www.agilent.com/chem.

   e. HPLC mobile phase filtering and degassing apparatus - Millipore Microfiltration Assembly, 47 mm, filtering with a type GV 0.2 micron filter.

   f. Analytical balance (± 0.0001 g) - Mettler Toledo AG204.

   g. Top loading balance (± 0.01 g) - Mettler Toledo PG5002.S Delta Range.

   h. Magnetic stirrer and stir bars Corning Stirring/Hot Plate - PC 420, and VWR, 5/16” Diameter x 2” stir bars, 58949-038.

   i. Meat grinder or food processor - Rival model 2100 M/2.

   j. Branson Sonifier 450 ultrasonic generator with a 1/4 inch micro tip or a mechanical blender such as an UltraTurrax No.T25 equipped with an S25N-10G dispersing rotor.

   k. Vortex-2 Genie test tube vortexer - Scientific Industries, Bohemia, NY 11716.

   l. Polypropylene centrifuge tubes - 50 mL conical, with closures, Blue Max 352070, 30 x 115 mm, 25/pk, Becton Dickerson, Franklin Lakes, NJ. USA, 07417-1886.

   m. Centrifuge -IEC Centra-8R centrifuge.

   n. Volumetric flasks - 50 and100 mL volumetric flasks.

   o. Volumetric flasks - 40 mL volumetric flasks - Kimble/Kontes, Vineland, NJ, 08360, Cat. No. 042029-0801.

   p. Test tubes - 16 x 100 mm test tubes with closures - CMS, Inc., Cat. Nos. 339-309 and 270-671, respectively (Fisherbrand Disposable Culture Tubes, Borosilicate glass, 16 x 125 mm, catalog #14-961-30).

q. Volumetric pipettes - Assorted Class A volumetric pipettes.

r. Graduated cylinders - 100 mL glass graduated cylinders.

s. Mixing cylinders - 100 mL graduated mixing cylinders, stoppered, CMS, Inc., Cat. No. 101-527.

t. Glass bottles - 1000 mL glass bottles.

u. Pasteur pipettes - Disposable glass Pasteur pipettes.

v. Membrane filters - Disposable 13 mm PVDF Membrane 0.22µm filters, HPLC Certified Minispike Outlet, Gelman Acrodisc PM4450T.

w. Small disposable syringes - Becton-Dickerson, 1 mL syringe, 309602.

x. Spatulas - Metal or Teflon coated.

y. N-Evap air/nitrogen stream evaporator - Meyer N-Evap Analytical Evaporator, Organomation Associates, Inc. South Berlin, MA 01549.

z. Vacuum apparatus for solid phase cartridges, or syringes for sample application to cartridges - Supelco Visiprep, Supelco, 595 North Harrison Road, Bellefonte, PA 16823-0048, 800-359-3041.

aa. Ultrasonic water bath - Branson model 2200, 125 watts.

bb. pH meter - Orion 611,ThermoOrion, 500 Cummings Center, Beverly, MA 01915-1699 USA.

cc. Solid Phase Extraction (SPE) Cartridges - Acidic alumina (Alumina A) approximately 2 g packing, Activity Grade I, Waters Sep-Pak Classic, No. 51800, Waters Sep-Pak Plus. No. 20500.

Note: Not all brands of acidic alumina produce acceptable results. See, Section K.1, Appendix, SPE CARTRIDGE TESTING.

dd. HPLC Column - C18 reversed phase such as Supelcosil LC-18-DB, 5 µm, 4.6 x 250 mm, Part No. 58355.

ee. Alternative HPLC column - C18 reversed phase HPLC column Beckman Ultrasphere IP, 5 µm, 4.6 x 250 mm, Part No. 235335.

C. REAGENTS AND SOLUTIONS

1. Reagents

   Note: An equivalent solution or reagent may be substituted.

   a. Water (H_2O) - HPLC grade or distilled, deionized.

   b. Methanol (MeOH) - HPLC grade.

   c. Acetonitrile (ACN) - HPLC grade.

   d. Ethyl acetate - HPLC grade.

   e. Glacial acetic acid - Reagent grade.

   f. 1-Pentanesulfonic acid sodium salt - HPLC grade.

   g. Sodium borate decahydrate - Reagent grade.

2. Solutions

   a. 1N sodium hydroxide solution:

      Dissolve 40 g sodium hydroxide in approximately 800 mL deionized water. Mix well. Cool to room temperature and dilute to 1 L.

      Note: Exothermic reaction. Recommend the flask be placed in an ice bath when initially dissolving the sodium hydroxide.

   b. Borate Buffer (0.025M, pH 10.3):

      Add 9.54 ± 0.05 g sodium borate to 900 mL of HPLC grade water in a graduated cylinder or glass bottle, and dissolve by mixing, add 1N sodium hydroxide (approximately 40 mL) until the pH is 10.3 ± 0.1. Dilute to 1 L with HPLC water. Store at room temperature.

      Note: Check buffer monthly. Acceptable range is 9.5 -11.0.

   c. HPLC Mobile Phase:

      Add 320 mL HPLC acetonitrile to 680 mL HPLC water. Then add 20 mL of glacial acetic acid and 0.87 ± 0.05 g 1-pentanesulfonic acid. Mix well, filter through a 0.45 µm filter if necessary, and degas.

   d. Sample Diluent (acetic acid - 2 percent v/v):

      Add 20 mL reagent grade glacial acetic acid to 980 mL HPLC water and mix well.

e. Mobile phase, for alternative HPLC column:

Prepare as directed in section C.2.c., except use 250 mL of acetonitrile and 750 mL of water.

D. STANDARDS

1. Source

Note: The analyst may prepare different volumes, provided the standard curve concentrations remain the same. The stock solution must be adjusted for purity during preparation.

a. Ractopamine HCl Reference Standard: Eli Lilly and Company, Indianapolis, Indiana 46285. Store at room temperature.

b. Ritodrine HCl Reference Standard: US Pharmacopeia Convention, 12601 Twinbrook , Rockville, Maryland 20852. (301) 881-0666. Catalog Number: 1604701. CAS number [23230-51-2]. Store at room temperature.

2. Reference Standard Preparation

a. Stock solution (1.00 mg/mL):

Prepare a ractopamine hydrochloride standard stock solution by adding 100 ± 0.1 mg of ractopamine hydrochloride reference standard to a 100 mL volumetric flask and diluting to volume with methanol.

(This solution is stable for three months at 2 - 8 °C.)

CAUTION: Wear gloves when handling reference standard. Do not inhale the dust of the primary reference standard.

b. Intermediate standard (10 µg/mL):

Pipet 1.0 mL of standard stock solution into a 100 mL volumetric flask and diluting to volume with sample diluent. Mix well. (This solution is stable for one month when stored at 2 - 8 °C).

c. Fortification standard (1.5 µg/mL):

Pipet 15 mL of 10 µg/mL intermediate standard solution into a 100mL volumetric flask and diluting to volume with sample diluent. Mix well. (These solutions are stable one month when maintained at 2 - 8 °C).

d. Ractopamine HCl external standard curve solutions (25, 50, 75, 150 and 300 ng/mL):

Prepare volumetric dilutions of the 10 µg/mL intermediate standard using sample

diluent. For 25, 50, 75, 150 and 300 ng/mL solutions make 250 µL to 100 mL, 500 µL to 100 mL, 750 µL to 100 mL, 1.5 mL to 100 mL and 3.0 mL to 100 mL volumetric dilutions, respectively. (These solutions are stable for one month when maintained at 2 - 8 °C).

3. Resolution Standard Preparation:

Prepare a solution containing ritodrine HCl and ractopamine HCl in the range of 10 to 25 ng/mL in sample diluent.

Note: Prepare this solution as needed to check column.

Note: Ritodrine HCl Reference standard is a USP standard so there are no corrections for purity.

a. Ritrodrine HCl stock solution (1 mg/mL):

Weigh 50 ± 0.1 mg of ritodrine hydrochloride reference standard into a 50 mL volumetric flask and dilute to volume with methanol.

b. Ritrodrine HCl intermediate standard (10 µg/mL):

Pipet 1.0 mL of standard stock solution into a 100 mL flask and dilute to volume with sample diluent. Mix well.

c. Resolution solution, mixed external standard (25 ng/mL):

Pipet 250 mL of the 10 µg/mL ritodrine HCl intermediate solution and 250 mL of the 10 mg/mL ractopamine HCl intermediate solution into a 100 mL flask and dilute to volume with sample diluent. Mix well.

E. SAMPLE PREPARATION

Preparation and Storage of Tissues

1. Initial processing includes grinding or blending of the tissues using a food grinder (or cryogenic grinding) to produce homogenous samples. Grind a minimum 500 g sample of tissue when possible.

2. Store all tissues at < -10 °C when not processing or sub-sampling. Ractopamine has been shown to be stable in frozen tissue for one year.

Note: Extreme care should be taken to make sure all tissue residue containing ractopamine is cleaned from glassware and other laboratory items in contact with samples and standards. It is recommended that disposable items be used whenever possible and that labware used with standards and other sources

containing high levels of ractopamine be kept separate from that used to prepare samples in the low ppb range.

F. ANALYTICAL PROCEDURE

1. Tissue Extraction

a. Weigh 10.0 ± 0.2 g of frozen or partially thawed ground sample tissue into a suitable container such as a 50 mL polypropylene centrifuge tube.

Note: Prepare blank and recovery samples at this time by weighing two 10 g blank tissues as part of the sample set. Prepare a 150 ppb recovery by adding 1 mL of 1.5 μg/mL fortification standard (D.2.c) to one of the tissue blanks.

b. Add 20 ± 1 mL of methanol to the sample.

c. Homogenize the tissue slurry for approximately one minute using an ultrasonic cell disrupter equipped with a 1/4 inch micro tip. Alternatively, the tissue may be blended for approximately one minute using a suitable blender to produce a uniform slurry. The probe must be cleaned between samples with methanol and water rinses, and given a final methanol rinse. A detergent may also be used to assist in cleaning mechanical homogenizers (e.g. UltraTurrix/Polytron). Let the sample stand at room temperature for 10 - 15 minutes to enhance solvent contact with tissue.

d. Centrifuge the tissue slurry at approximately 3000 rpm (approximately 1500 g) for 10 minutes. Exact speed and centrifugal force is not critical provided a good sediment pack is obtained. Refrigeration may be used, but is not necessary.

e. Decant the supernatant into a 100 mL graduated mixing cylinder or other appropriate graduated glassware.

f. Add a second 20 mL of methanol to the tissue, vigorously suspend the centrifuged pack with a spatula, centrifuge as in step d above, and add the second supernatant to the first. Combined extracts will be cloudy.

g. Repeat Step f, adding the third supernatant to the first and second.

h. Dilute the combined supernatants to 60 ± 1 mL with methanol and mix well.

Note: This is a suitable stopping point. Extracts may be stored for 7 days at 2 - 8 °C.

i. Pipette 8.0 ± 0.1 mL aliquot of the combined supernatant into a 16 x 100 mm test tube. Evaporate the sample to less than 0.5 mL under air or nitrogen at 49 ± 2 °C.

Note: Dry the sample aliquot until less than 0.5 mL remains. This is sufficient to remove most of the methanol. A thin film of oily residue will remain on the side of

the test tube. Time required is approximately 30 - 45 minutes using a N-Vap.

j. Add 2 mL of borate buffer and mix briefly. A repipet dispenser or disposable pipette is sufficiently accurate to use for the buffer addition.

2. Liquid/Liquid Extraction

a. Add 7 mL of ethyl acetate to the test tube containing the sample and vortex for at least 30 seconds. A repipet dispenser or disposable pipette is sufficiently accurate to use for the ethyl acetate addition.

b. Centrifuge the tube for 5 minutes at 2000 rpm (approximately 560 g).

c. Transfer the upper layer (ethyl acetate) into a clean 16 x 100 mm test tube using a disposable pipette or other suitable means, taking care to remove as much of the layer as possible without removing any of the lower fraction (borate buffer).

d. Repeat steps 2.a. and 2.b. with fresh ethyl acetate. To save time, do not transfer upper layer. Proceed to solid phase extraction.

3. Solid Phase Extraction

a. Wet an acidic alumina SPE cartridge using approximately 5 mL of ethyl acetate, let the solvent drain to the surface of the cartridge. Flow rate is not important.

b. Transfer the second ethyl acetate fraction from step 2.d. to the cartridge; follow with the first ethyl acetate fraction from step 2.c. Drain the combined ethyl acetate fractions to the surface of the SPE cartridge at a flow rate of approximately 2-4 mL/minute using vacuum if necessary. Discard the cartridge effluent.

c. Wash the cartridge with approximately 5 mL of ethyl acetate at approximately the same flow rate as the sample application and stop the flow when the liquid reaches the surface of the cartridge packing. Discard the cartridge effluent.

d. To elute ractopamine from the cartridge, add approximately 10 mL of methanol to the cartridge and collect the effluent in a 16 x 100 mm test tube or equivalent vessel. Force the liquid completely from the cartridge using either pressure or vacuum if necessary. Flow rate of methanol should be no greater than approximately 5 mL/minute.

Note: This is a suitable stopping point. The methanol effluent may be stored for 14 days at 2 - 8 °C if needed, before continuing with the method.

e. Evaporate the sample to dryness using an air or nitrogen stream and a water bath or heater set at 49 ± 2°C.

f. Dissolve the sample in 1.0 mL of sample diluent by swirling the tube or vortexing vigorously for 15 - 30 seconds, and sonicating for approximately 15 seconds in an

ultrasonic water bath.

g. Filter the sample through a PVDF 0.22 µm x 13 mm syringe filter using a small disposable syringe and collect the filtered sample in a HPLC autosampler vial.

Note: This is a suitable stopping point. Samples may be stored for 4 days at 2 - 8 °C if needed, until the sample analysis is completed.

4. Sample Analysis

Note: Instrument conditions may be modified and the alternative column used provided changes are documented and system suitability criteria are met.

a. Operating Conditions

i.	Column:	4.6 mm i.d. x 25 cm Supelcosil LC-18-DB, (guard column may be used)
ii.	Excitation:	226 nm
iii.	Emission:	305 nm
iv.	Flow Rate:	1.0 mL/minute
v.	Injection Volume:	100 µL
vi.	Column Temperature:	Ambient (20 -25 ° C)
vii.	Run Time:	10 minutes
viii.	Mobile Phase:	Refer to section C.2.c.

b. Test for System Suitability

i. System suitability items: ii, iii, and iv should be checked as part of each sample run. Item v should be checked whenever the column is replaced or when any significant changes are made to the instrument setup. If system suitability requirements are not met refer to Item vi for corrective action. Inject standard and verify ii, iii, and iv.

ii. The signal/noise ratio of the 25 ng/mL standard should be at least 5.

iii. The ractopamine peak should be well resolved from the solvent front and elute between 4 - 8 minutes. The retention time of ractopamine should not vary more than 30 seconds among the samples and standards within a set.

iv. Ractopamine must be baseline resolved from ritodrine. See Figure 1, Section K.2, for example chromatogram.

v. If the signal/noise ratio does not meet the specification in ii, the detector may not be adequate to analyze samples at levels required by this method. If the conditions specified in iii, and iv are not met, the mobile phase may need to be modified or the column needs to be replaced.

Note: Decreasing acetonitrile concentration in the mobile phase increases the resolution of ractopamine from ritodrine.

c. Analyze Sample Set

i. If above items are satisfactory, inject the standard and samples. It is advisable to inject a complete set of standards at the beginning and at least one standard at the end of each sample set. A recommended scheme is as follows:

Resolution standard (optional)
Set of standards
Recovery
Blank
Samples
At least one standard vial (to check for any change in retention time and response).

Note: It is recommended that the column be flushed with a strong solvent/water mixture (acetonitrile or MeOH/water, 80:20) after the end of the analytical batch to remove residual matrix from the column.

ii. If the peak area for ractopamine HCl in a sample exceeds the high end of the standard curve, the final extract should be diluted and re-injected along

with one standard curve set. Another option is to take a smaller aliquot of the initial methanol extract and reprocess the sample. If the amount found exceeds known SPE cartridge capacity, the sample should be reprocessed using a smaller aliquot (step F.1.i) in order to assure a consistent recovery.

Note: The capacity of Waters Sep-Pak Plus Alumina A cartridges has been verified with 150 ppb recovery samples using 4x the recommended aliquot of the combined supernatant (step F.1.i).

5. Sample Chromatograms

See Figures 1and 2 in section K.2

G. CALCULATIONS

a. Measure the HPLC peak area for ractopamine HCl in the standard and sample solutions. Construct a linear standard curve using all of the standard responses. Note: Standard curve must be linear (have a correlation coefficient (r value) ≥ 0.995) over the concentration range used for quantitation.

b. From the standard curve; calculate the concentration in ng/mL of each of the extract solutions. (Refer to section K.1. in the event acceptable recovery and blank control results are not obtained.)

c. Calculations

The concentration of ractopamine can be calculated using the following equation:

ppb Ractopamine HCl = $\frac{[(A-B)xE]}{(CxF)}$

A = HPLC peak area of sample injection

B = Intercept from the calibration curve

C = Slope of the calibration curve (area/ng/mL)

E = Total volume (mL) = (Initial volume/aliquot volume) x final volume

F = Weight of tissue sample (g)

Note: Area response should be linear with respect to concentration of ractopamine over the range of 25 to 300 ng/mL for standards and 25 to 300 ppb.

H. HAZARD ANALYSIS

1. Method Title — Determination of Ractopamine Hydrochloride in Swine and Bovine Liver and Muscle Tissue by High Performance Liquid Chromatography.

2. Required Protective Equipment —Safety eyewear, protective gloves, and lab coat.

3. Hazards

Reagent	*Hazard*	*Recommended Safe Procedures*
Glacial acetic acid	Strong acid	Wear protective equipment, avoid contact with skin.
Ractopamine HCl	Eye irritant and exposure may increase heart rate.	Wear protective equipment, avoid breathing powder.
Ritodrine HCl	Irritant, fast or irregular heartbeat, nausea, shortness of breath.	See ractopamine HCl above.
Methanol, Ethyl Acetate	Flammable	Keep in well-closed containers in a cool place and away from fire. Use it in well-ventilated hood.

4. Disposal Procedures

Reagent	*Hazard*	*Recommended Safe Procedures*
Glacial acetic acid	Strong acid, burns	Collect waste in a tightly sealed container and store away from non-compatibles in a cool, well ventilated, acid liquid storage area/cabinet for disposal in accordance with local, State, and Federal regulations.
Ractopamine HCl	Eye irritant and exposure may increase heart rate.	Collect waste in a tightly sealed container and store in a cool, well-ventilated storage area/cabinet for disposal in accordance with local, State, and Federal regulations.
Ritodrine HCl	Irritant, fast or irregular heartbeat, nausea, shortness of breath.	See ractopamine HCl above.
Organic solvents	Flammable	Collect waste in a tightly sealed container and store away from non-compatibles in a cool, well ventilated, flammable liquid storage area/cabinet for disposal in accordance with local, State, and Federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range*	*Acceptable Recovery*	*Acceptable Repeatability (CV)*
Ractopamine HCl	25 – 300 ppb	60 – 115 %	≤20 %

Acceptable correlation coefficient for standard curve: ≥0.995.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
Sample weight	10.0 ± 0.2 g
Methanol	20 ± 1 mL
Combined methanol supernatant	60 ± 1 mL
Aliquot Volume	8 ± 0.1mL
Water bath temperature	49 ± 2 °C

3. Readiness To Perform (FSIS Training Plan)

   a. Familiarization

      i. Phase I: Standards - Duplicate standard curve on each of 3 consecutive days, which will include the following:

         (a) 0 ng/mL

         (b) 25 ng/mL

         (c) 50 ng/mL

         (d) 75 ng/mL

         (e) 150 ng/mL

         (f) 300 ng/mL

      ii. Phase II: Fortified Samples - Eight recovery curves, two in porcine liver, two in porcine muscle, two in bovine liver and two in bovine muscle, on four separate days at 0, 25, 50 150, and 300 ppb.

For example:

Set 1. One recovery curve in porcine liver and one recovery curve in porcine muscle.

Set 2. A second recovery curve in porcine liver and a second recovery curve in porcine muscle.

Set 3. One recovery curve in bovine liver and one recovery curve in bovine muscle.

Set 4. A second recovery curve in bovine liver and a second recovery curve in bovine muscle.

Note: Phase I and II may be performed concurrently. When running each set of two recovery curves, different species or tissues (liver or muscle) shall be analyzed. For example, a recovery curve in porcine muscle and a recovery curve in bovine muscle would also be an acceptable set for one day. Two recovery curves in porcine muscle extracted in the same set would not.

iii. Phase III: Check samples. given by the supervisor or designee.

(a) 6 unknowns fortified between 25 - 300 ppb, one of which is ND.

(b) Approval from the Supervisor of Record and the Laboratory Quality Assurance Manager (QAM) is required to commence official analysis.

b. Acceptability criteria.

Refer to section I.1. above.

4. Intralaboratory Check Samples

a. Frequency:

i. One sample per week per analyst as samples analyzed.

ii. Records are maintained.

b. Acceptability criteria:

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability
   a. Matrix: liver, muscle
   b. Sample receipt size: approximately 500 g
   c. Condition upon receipt: not spoiled or rancid
   d. Sample storage:
      i. Time: 2 months (This is the prepared sample.)
      ii. Condition: Frozen <-10°C.

6. Sample Set

   Note: Each sample set must include:
   a. Blank tissue.
   b. Blank tissue fortified with 1 mL of the 1.5 μg/mL fortification standard. (Fortification solution D.2.c. is equivalent to 150 ppb).
   c. Samples

7. Sensitivity
   a. Lowest detectable level (LDL): To be determined.
   b. Lowest reliable quantitation (LRQ): 25 ppb
   d. Minimum proficiency level (MPL): 25 ppb

J. WORKSHEET

The worksheet, on the following page, is only an example and can be removed for photocopying.

RACTOPAMINE DETERMINATIVE FORM

Analyst:
Date Started: _____
Date Completed: _____
Set Number: _____
Species: _____

Reviewed By (Init./Date) _____

N-Evap (Step F.1.i) Temperature (49 ± 2°C) _____

N-Evap (Step F.3.e) Temperature (49 ± 2°C) _____

HPLC Conditions	Run time :	
	Injection vol. :	
	Pressure :	
	Flow rate :	
	Column Temp:	
	Mobile Phase:	
	Column Type:	
Detector Conditions	excitation (λ):	226 nm
	emission (λ):	305 nm

Sample Analysis Data

Sample No.	Lab. No.	Form No.	Tissue Type (02 or 03)	Sample Wt. (10.00 ± 0.20 g)	Methanol (20 ± 1 mL)	Methanol Dilution Volume (60 ± 1 mL)	Aliquot Volume (8.0 ± 0.1 mL)
1	Recovery						
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

REMARKS:

Equipment/Reagents used	ID #
Micropipet	
N-Evap	
Centrifuge	
HPLC	
Methanol	
Borate Buffer	
Ethyl Acetate	
Sample diluent	
SPE-Cartridge	
Seal Wash	
Mobile Phase	
Balance	

K. APPENDIX

1. SPE cartridge testing

In the event that acceptable standard recovery (> 60 percent) and control results are not attainable using the method; the following steps may be taken to determine the suitability of the acidic alumina SPE cartridges.

Note: 2 g acidic alumina activity I from Waters, Alltech Isolute, and ICN proved to be acceptable using this test.

a. Preparation of Test Solutions

i. Ractopamine HCl fortification solution (0.2 µg/mL):

Pipet 2 mL of the 10 µg/mL Intermediate solution into a 100 mL volumetric flask and dilute to volume with sample diluent. Mix well. (This solution is stable for one month when stored at 2 - 8 °C.)

ii. Ractopamine HCL external standard (2.5 ng/mL):

Pipet 10 mL of the 25 ng/mL solution into a 100 mL volumetric flask and dilute to volume with sample diluent.

b. Test Procedure 1

i. Fortify 2 mL of Borate Buffer with 0.1 mL of the 0.2 µg/mL fortification standard (20 ng).

ii. Perform steps F.2.a through F.3.g and analyze as described in the method.

iii. At least 85 % (17 ng) of the fortification should be recovered.

iv. If 85% is not recovered, a new source of acidic alumina should be tested.

c. Test Procedure 2.

The suitability may further be evaluated using the following procedure, which will determine the SPE performance in the presence of control and fortified liver tissue extracts.

i. Prepare control extracts of tissue following steps F.I.a through j.

ii. Fortify the desired number of samples with 0.1 mL of a 0.2 µg/mL fortification standard (20 ng).

Note: 20 ng fortified at the borate buffer stage is equivalent to 15 ppb in tissue.

iii. Perform steps F.2.a through F.3.g and analyze as described in the method.

iv. At least 80 % (16 ng) of the fortified analyte should be recovered.

v. The area of the tissue blank at the retention time of ractopamine should be less than 20 % of the area obtained when injecting 100 µl of the 2.5 ng/mL external standard.

vi. If 80 % is not recovered, a new source of acidic alumina should be tested.

2. Chromatograms

Figure 1. Resolution of ractopamine and ritodrine (2.5 ng each injected on column) using a Supelcosil LC-18-DB column.

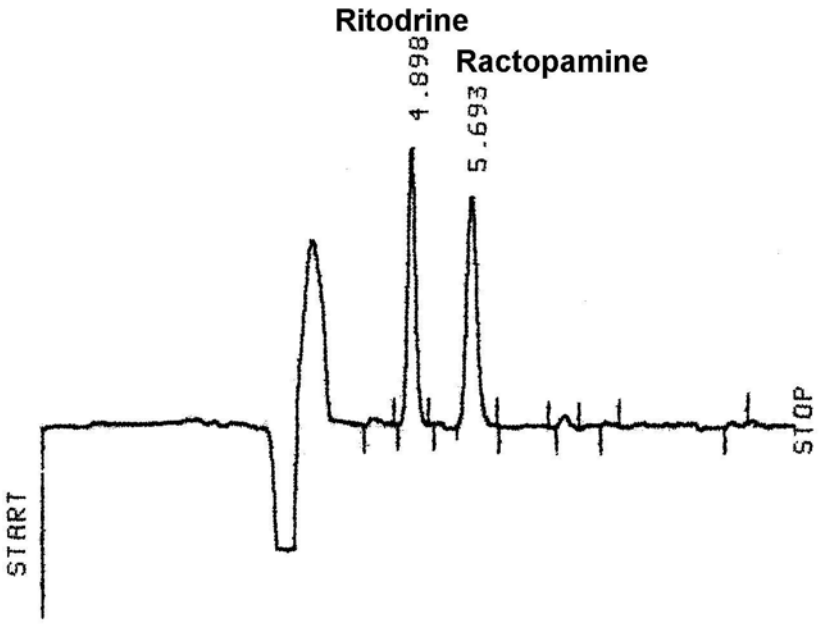
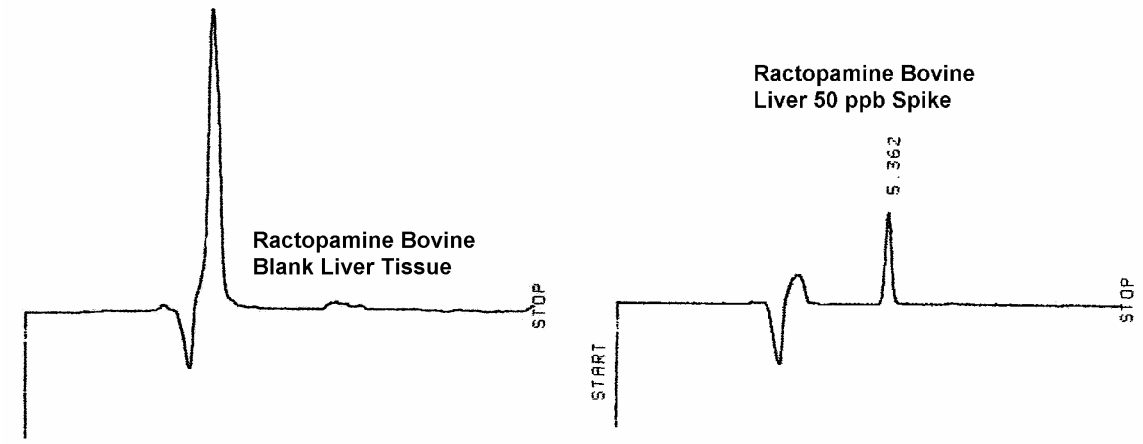


Figure 2. Chromatograms showing blank and spiked tissues respectively



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Approved By:	Date Approved:
Kathy Holland	4/03/03
Tom Mallinson	4/03/03
Terry Dutko	3/2703
Jess Rajan	3/28/03
Phyllis Sparling	3/27/03
Charles Pixley	4/07/03